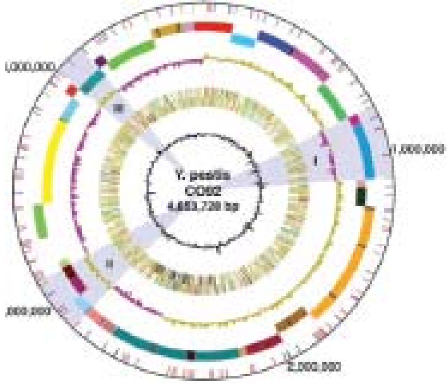
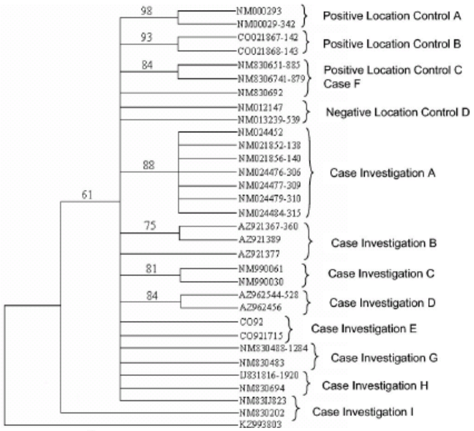
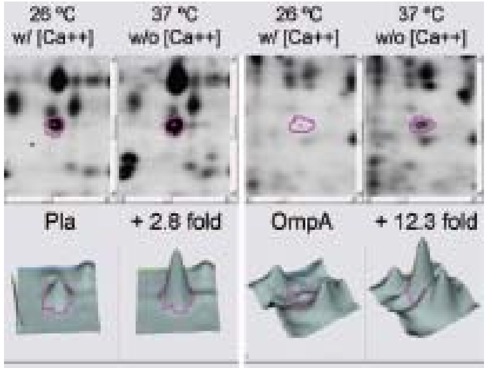
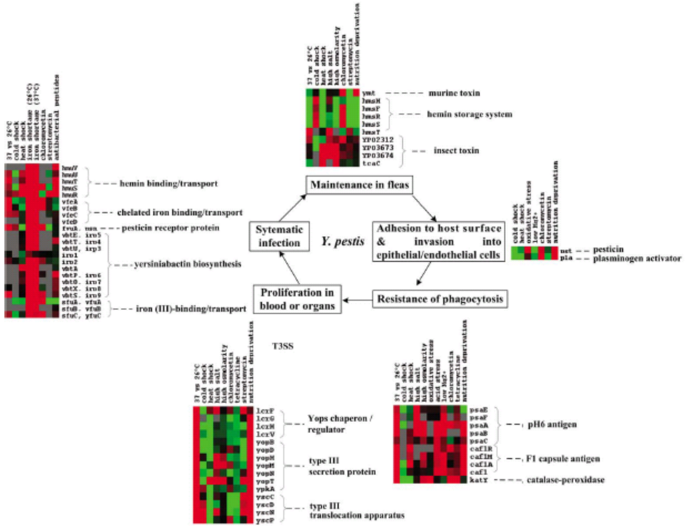
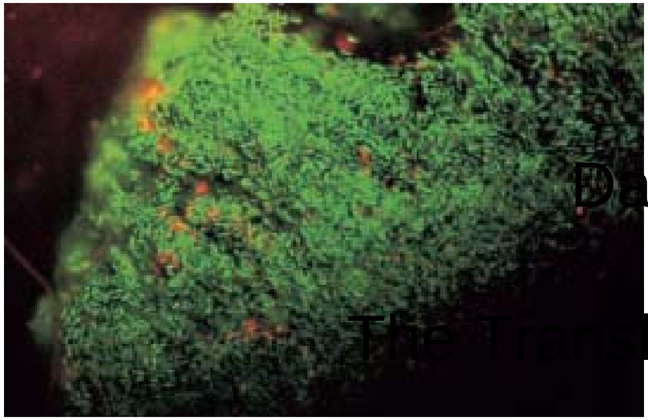


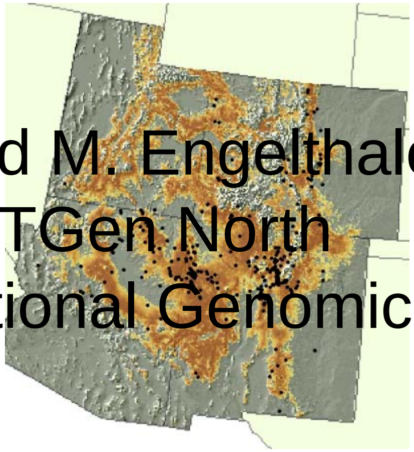
Plague in the 21st Century

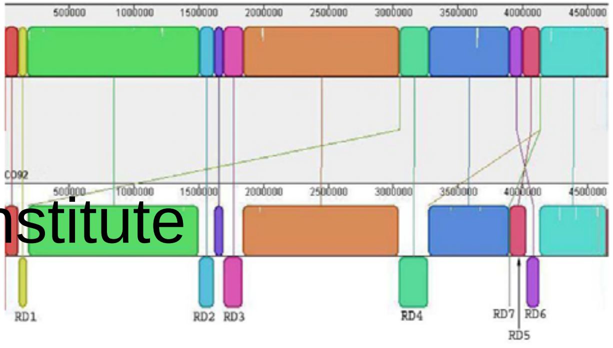








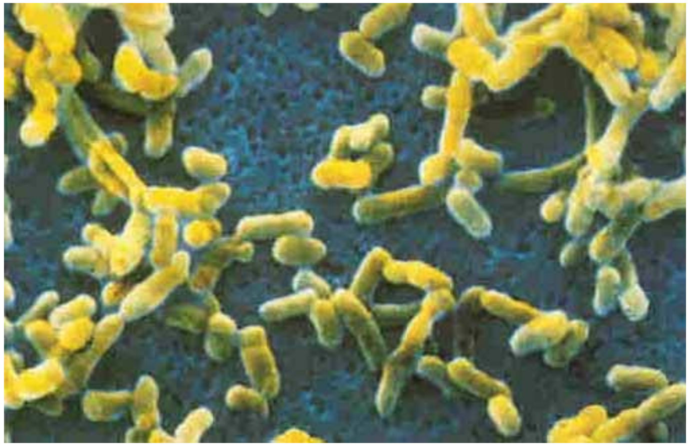




David M. Engelthaler
TGen North
The Translational Genomics Institute

The Pestilent Triumvirate

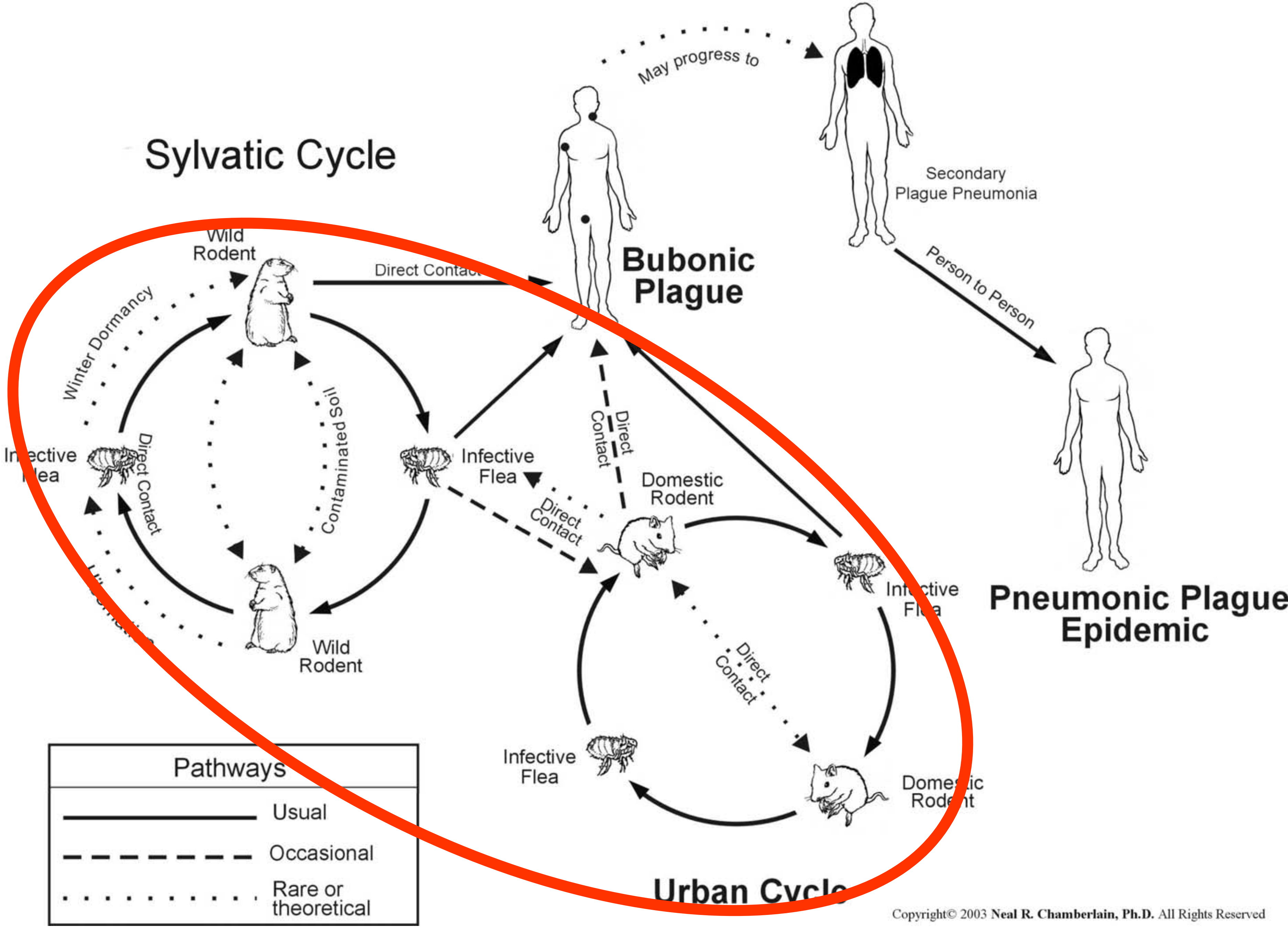
- *Yersinia pestis* – Extremely virulent gram-neg bacterium



- *Xenopsylla cheopis* – Unexcelled plague vector

- *Rattus* – Widespread commensal rodent; highly susceptible to plague

Plague Natural History


Sylvatic Cycle
May progress to
Secondary
Plague Pneumonia
Bubonic
Plague
Person to Person
Wild
Rodent
Direct Contact
Winter Dormancy
Contaminated Soil
Direct Contact
Infective
Flea
Wild
Rodent
Direct
Contact
Direct
Contact
Domestic
Rodent
Direct
Contact
Pneumonic Plague
Epidemic
Infective
Flea
Pathways
Usual
Occasional
Rare or
theoretical

Urban Vector/Host

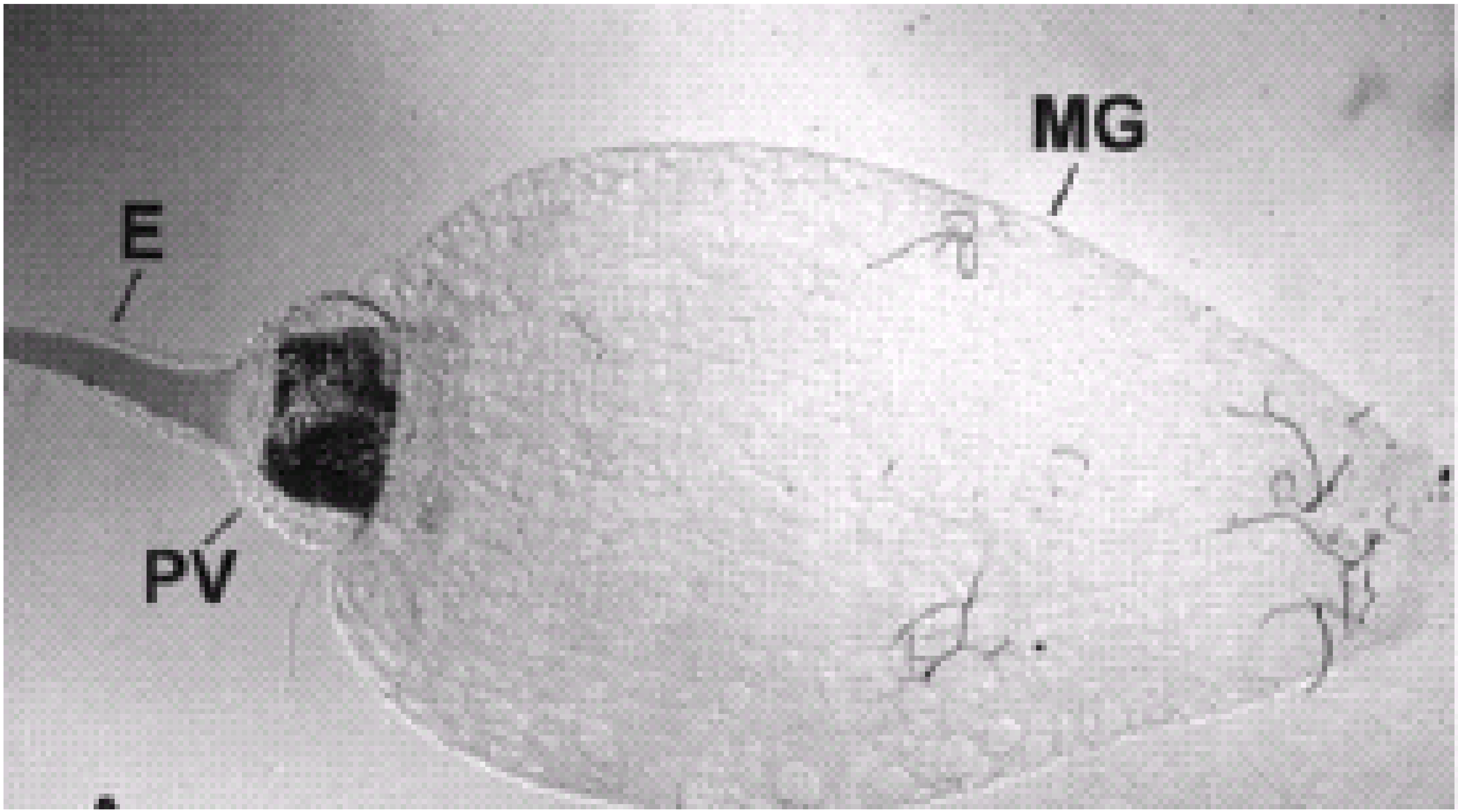
Sylvatic Vector/Host

Transmission by Fleas

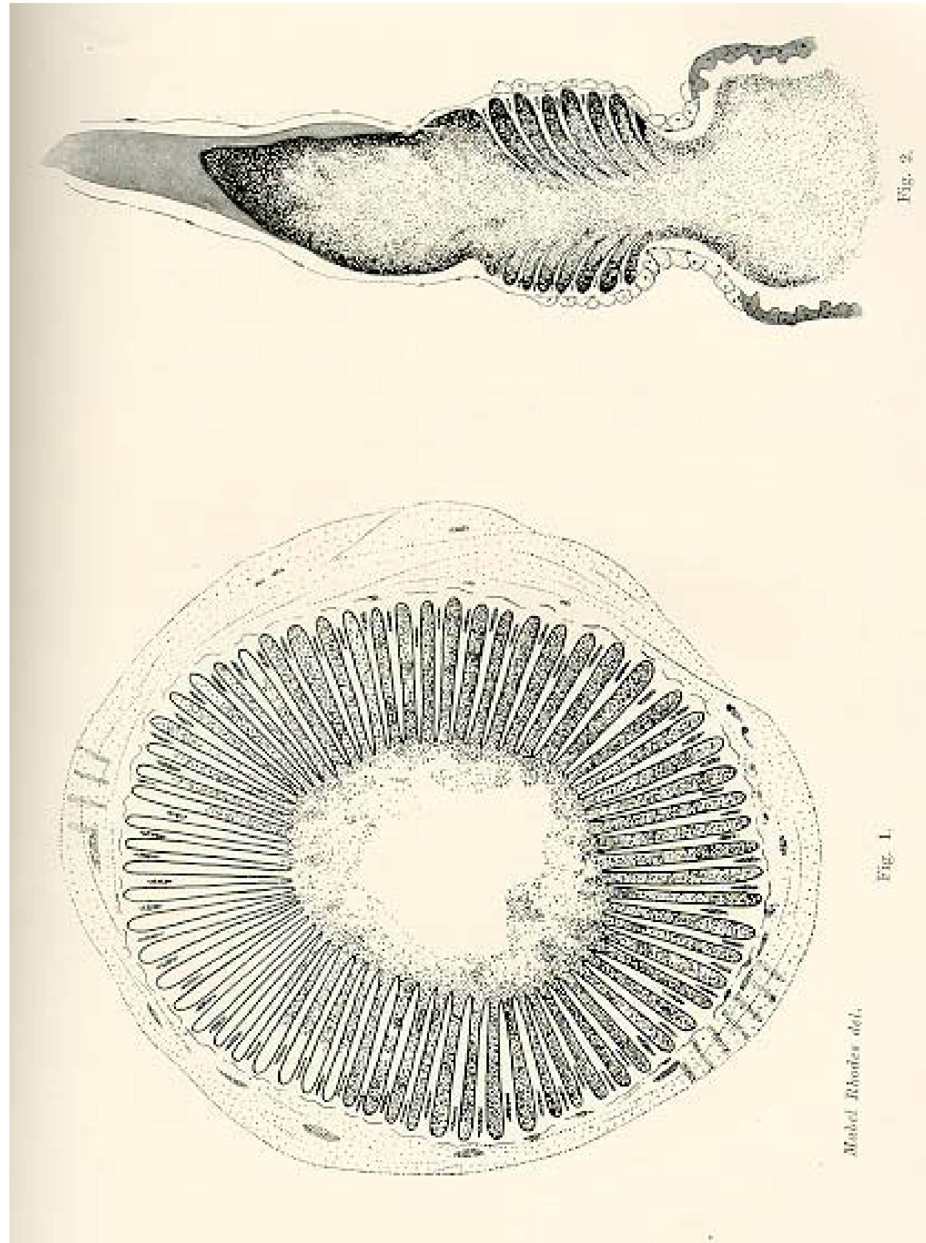
The Plague Dogma: Blocking in Fleas

- Bacot and Martin – 1914
- Infected *X. cheopis* develop “jelly-like masses of a brown color”(plague colonies) in their midguts and proventriculi
- Occlusion of the proventriculus (block formation)
- Starving, blocked fleas repeatedly attempt to draw blood into the foregut – distends esophagus
- *Y. pestis*-infected blood flushes back into the feeding site

The Flea Gut

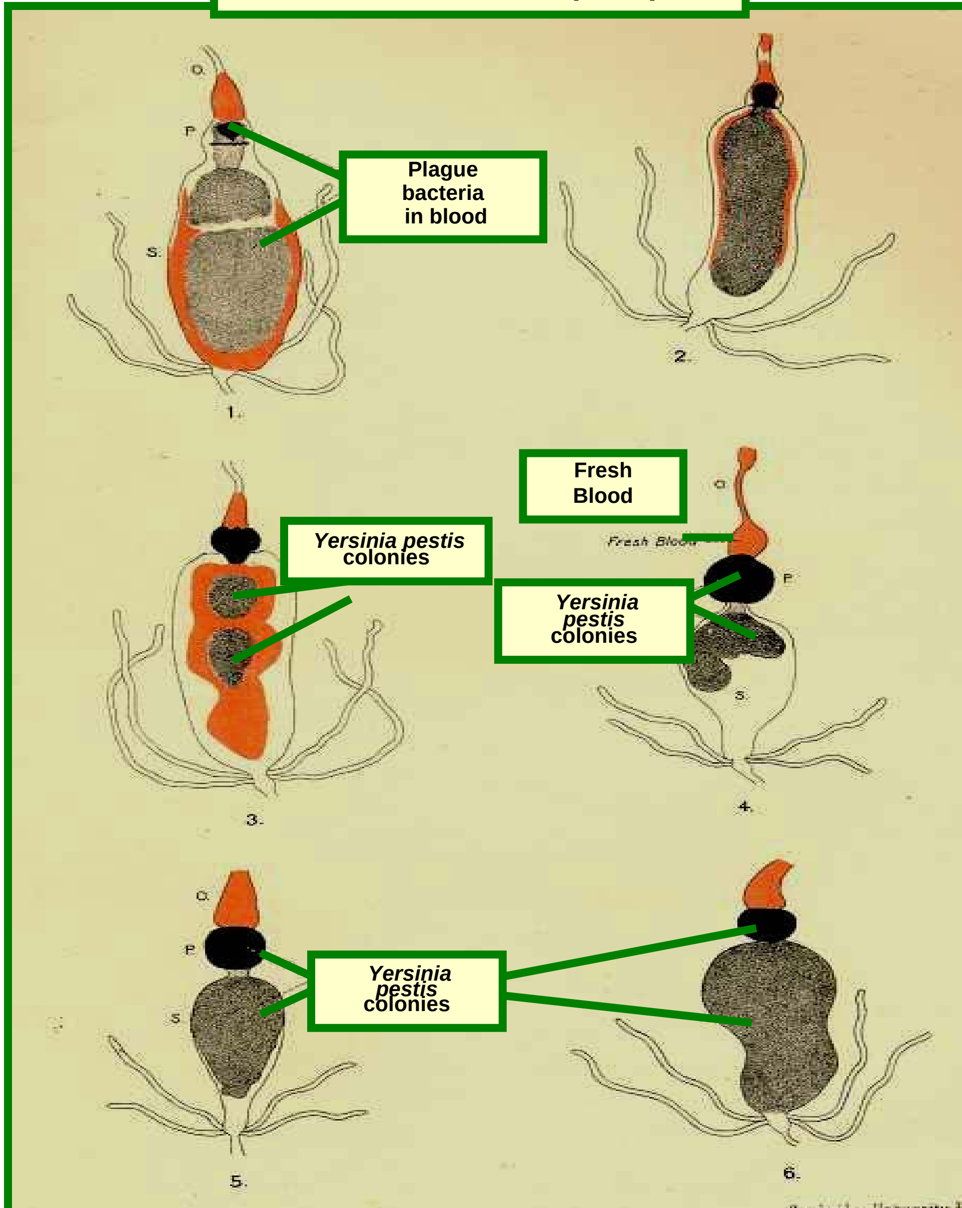


The Proventriculus



Blocking and Survival of *Yersinia pestis* in Fleas

Bacot and Martin (1914)



Blocked *Xenopsylla cheopis*

Vector-Pathogen Dynamics

- Since the “Plague Dogma” was originated several studies have elucidated the relationship between fleas and pestis

What we know:

- Fleas need to be blocked to transmit
- Fleas become infectious (“blocked”) after some period of time (extrinsic incubation period)
- Blocked fleas die relatively soon after blockage, from starvation/dehydration
- Different fleas have different vector efficiencies
- Y. pestis has specific virulence and transmission factors

Quantifying Pestis

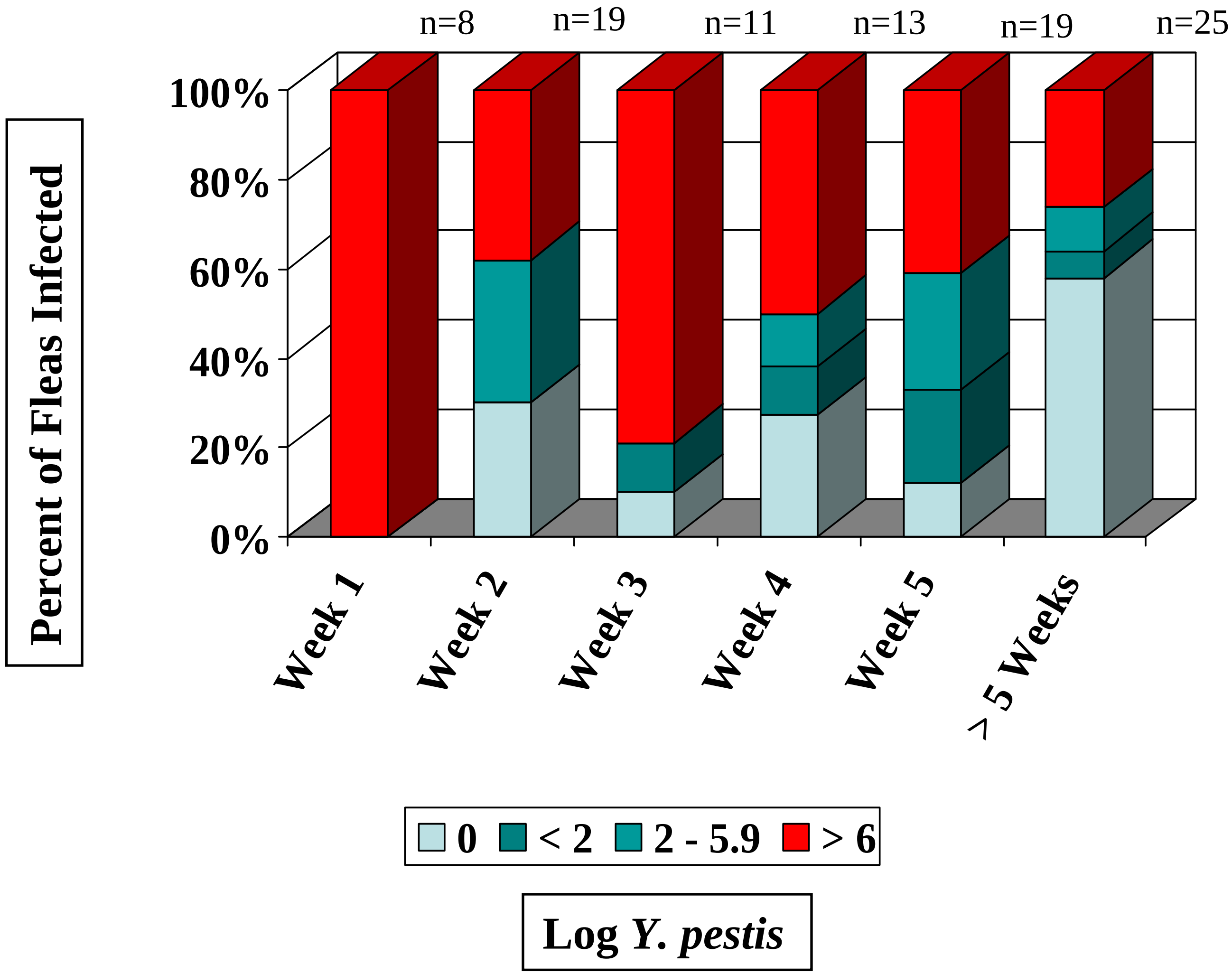
- Hinnebusch et al. (1998-2002), using quantitative competitive PCR estimated that a bacterial load of 10^6 cells was needed for fleas to become blocked (*X. cheopis*)

- Surmised that this could be used to estimate the infectiousness of fleas collected during epizootics
- Early testing of fleas could provide info on public health risk of epizootics

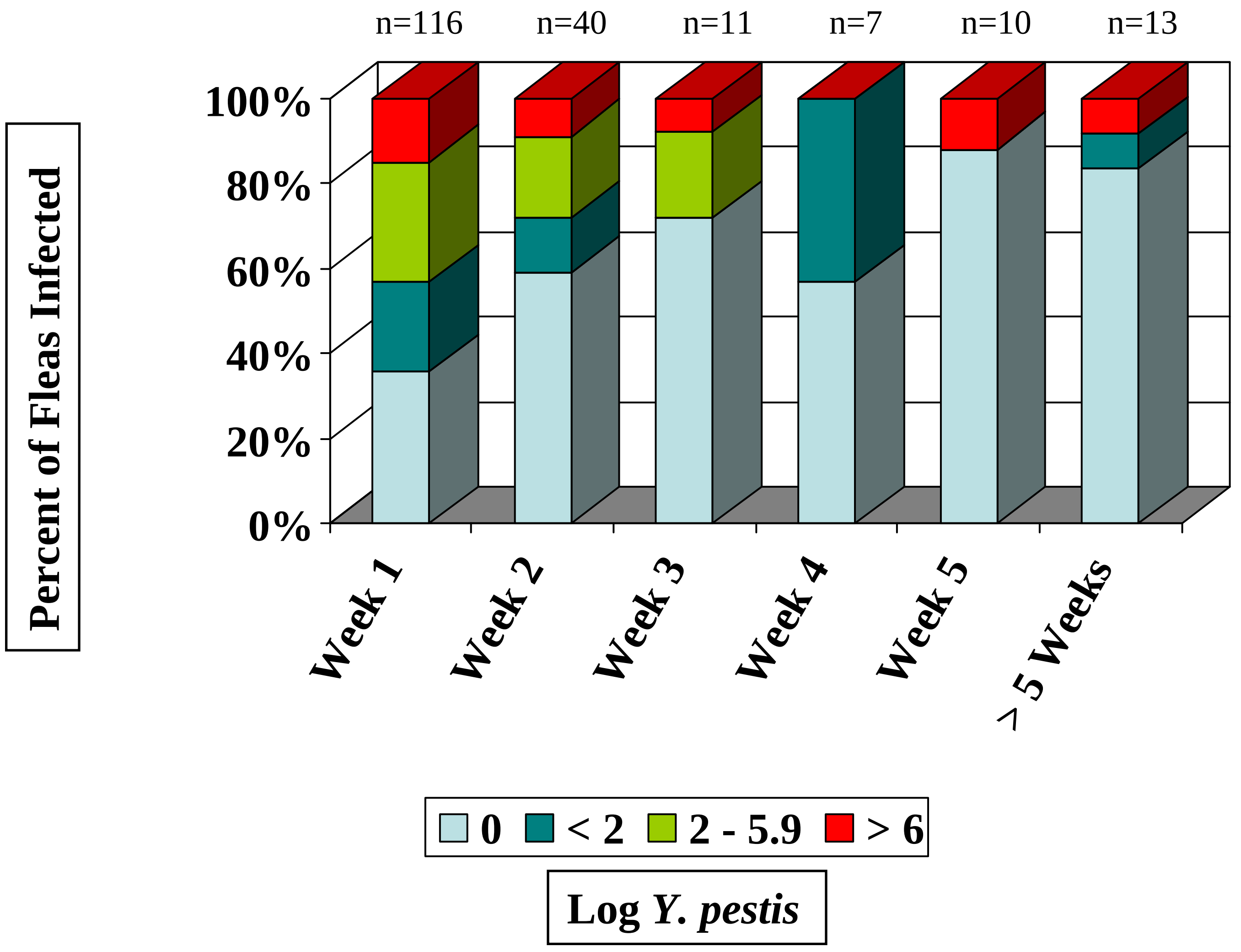
Some of My Research Questions

- Can QCPCR actually provide important information regarding infectiousness of fleas?
- Most experiments done with *X. cheopis* – Is this really typical for transmission in wild rodent-flea cycles?
- How can we explain differences in vector competency?
- How important is transmission by partially blocked or unblocked fleas?
- How important are fleas as reservoirs of *Y. pestis* infection?

Bacterial Loads in *Xenopsylla cheopis* by Week Post-Infection



Bacterial Loads in *Oropsylla montana* by Week Post-Infection



Transmission Studies

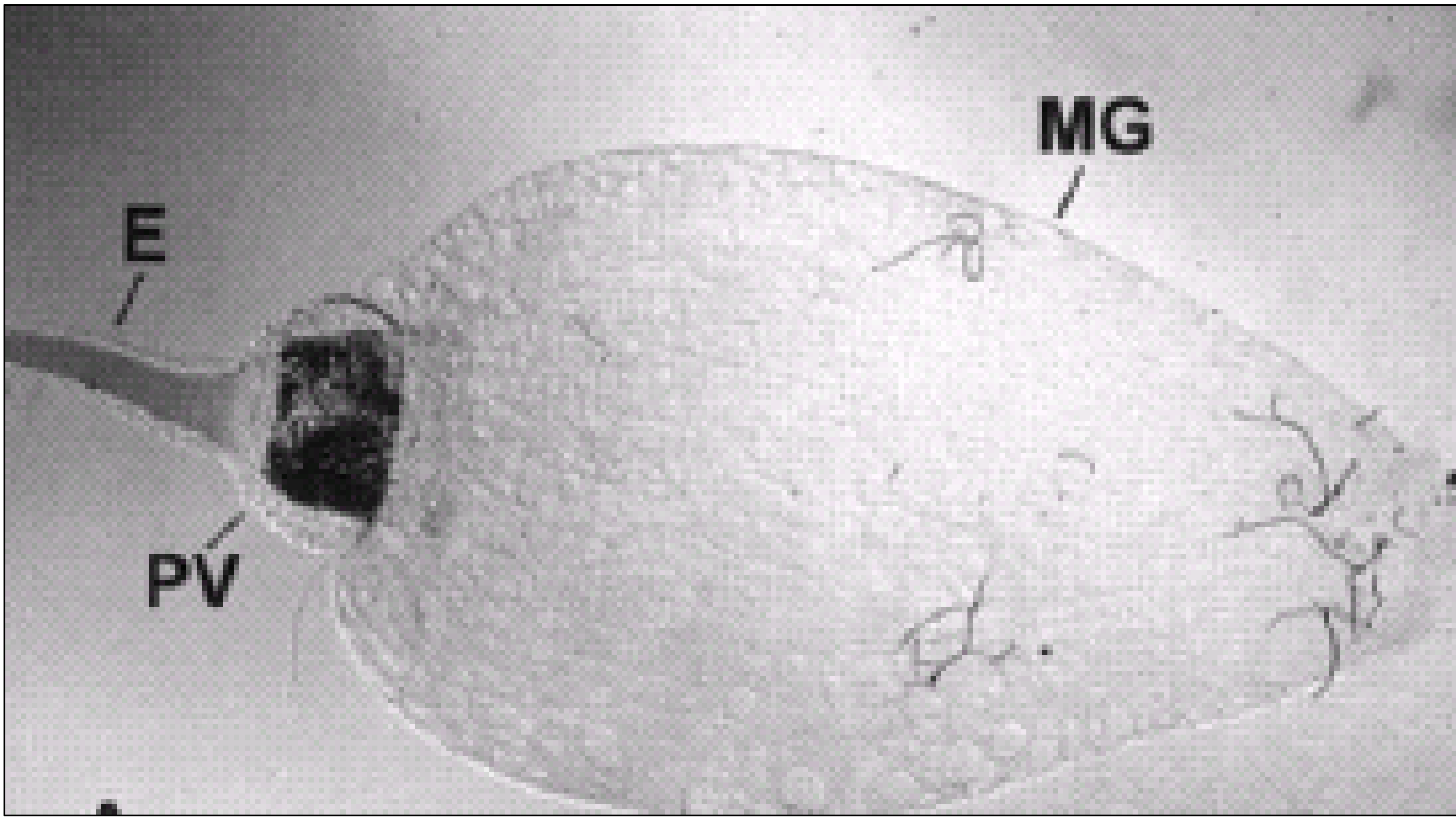
	Visibly Blocked Only	Transmitted Only	Both	Total
***X. cheopis* (n=95)**				
%	7	14	2	**23**
No. pestis/flea	10^6.7	10^7.1	10^7.1	
EIP* (range)	20 (16-23)	16 (6-32)	17 (15-18)	
***O. montana* (n=196)**				
%	0	2	0	**2**
No. pestis/flea	-	10^6.2	-	
EIP	-	**23 (4-37)**	-	

*EIP = Extrinsic Incubation Period – the number of days post-infection until transmission

Colonization Location in Fleas

- Blockage requires *pestis* colonization in proventriculus
- Can a large colony can grow in midgut and eventually occlude the proventriculus?
- Does *pestis* colonize in the same locations for both *X. cheopis* and *O. montana*?
- Is it possible to quantify bacterial loads in proventriculus vs. midgut?

The Flea Gut



Colonization Location in *X. cheopis* and *O. montana*

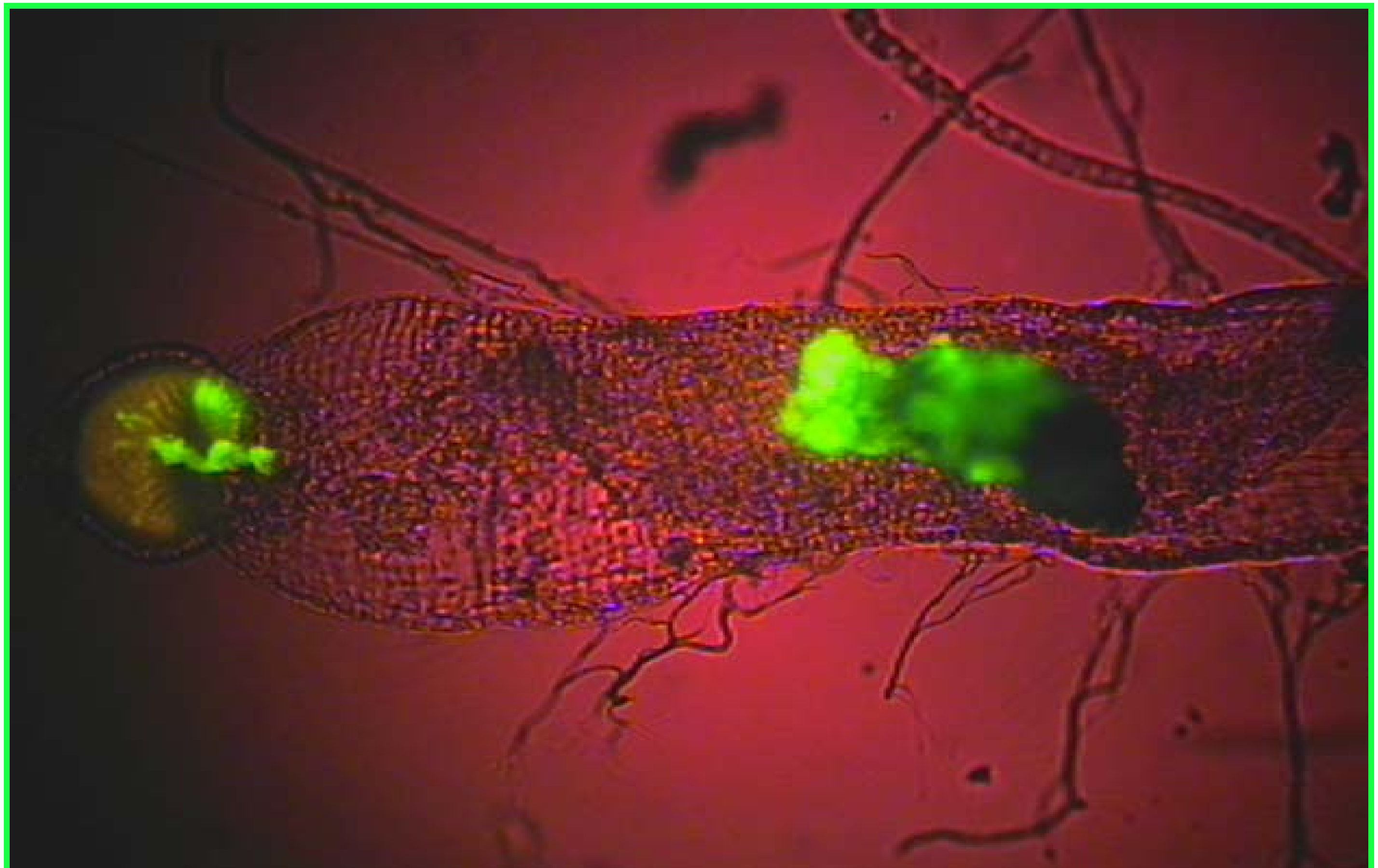
- Combined infection of the proventriculus and midgut is more common in *X. cheopis* than *O. montana* (81.3% vs. 16.7%)
- In no case was the proventriculus solely infected
- Both species can have heavy midgut loads (>10^6 *Y. pestis*) without proventricular infection and then fail to transmit

Visualizing *Y. pestis* Infection in Flea Gut

- “Dark mass” does not equal plague colony
- Transform *Y. pestis* with the Green Fluorescent Protein Gene
- Produce Green Glowing plague –
  - Y. pestis::pEGFP
  - Easily visualize colonies under UV light

Visualizing *Y. pestis* in the Flea

Flea midgut infected with *Yersinia pestis* transformed with Green Fluorescent Protein (GFP)



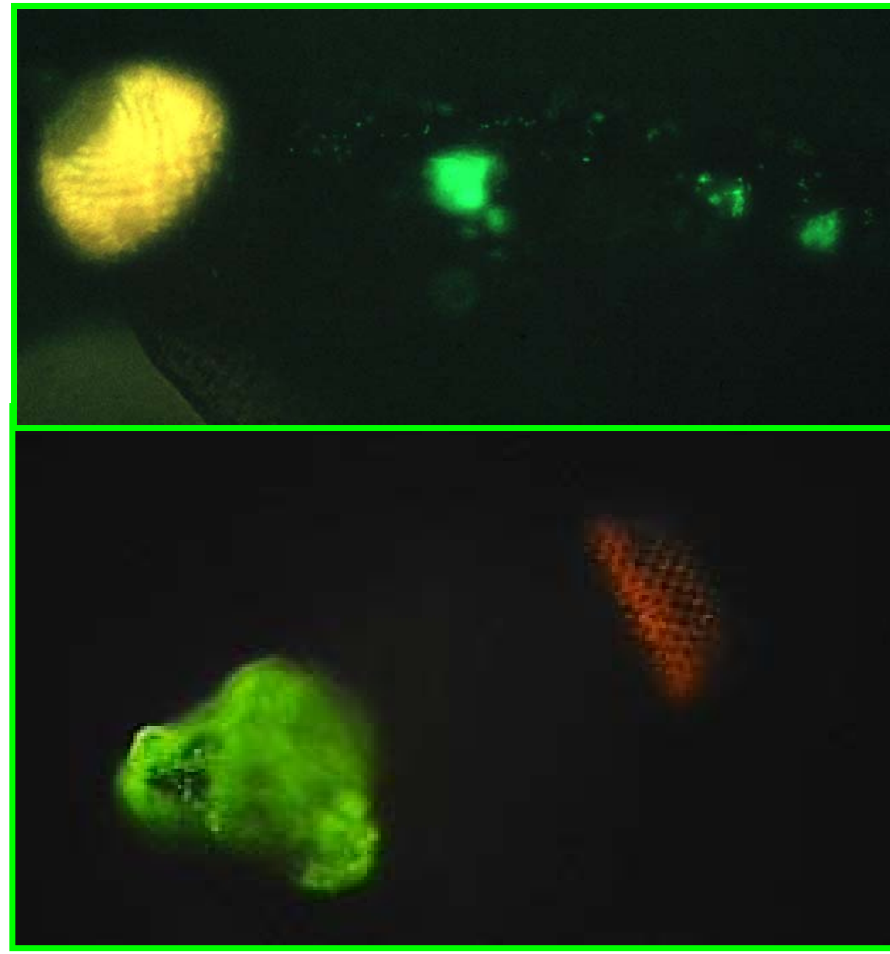
Xenopsylla cheopis midgut and proventriculus infected with *Y. pestis*

- *Y. pestis* simultaneously colonizes midgut and proventriculus
- Similar to the QC-PCR results
- Helps further explain why *X. cheopis* is such a effective vector

(Engelthaler et al. 2000)

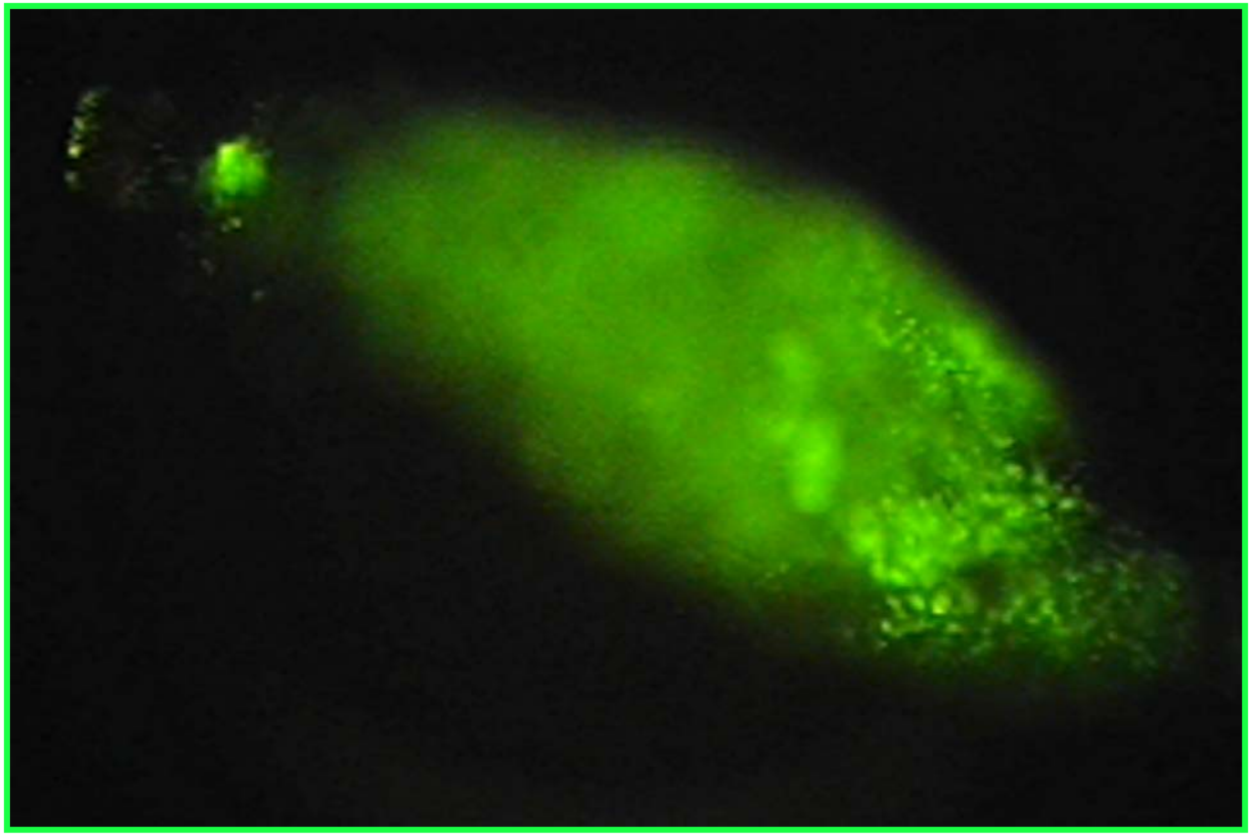
Oropsylla montana midgut infected with *Yersinia pestis*

- Colonies typically seen in the midgut only
- Again, validating the QC-PCR findings
- And further provides evidence why *O. montana* are relatively inefficient vectors

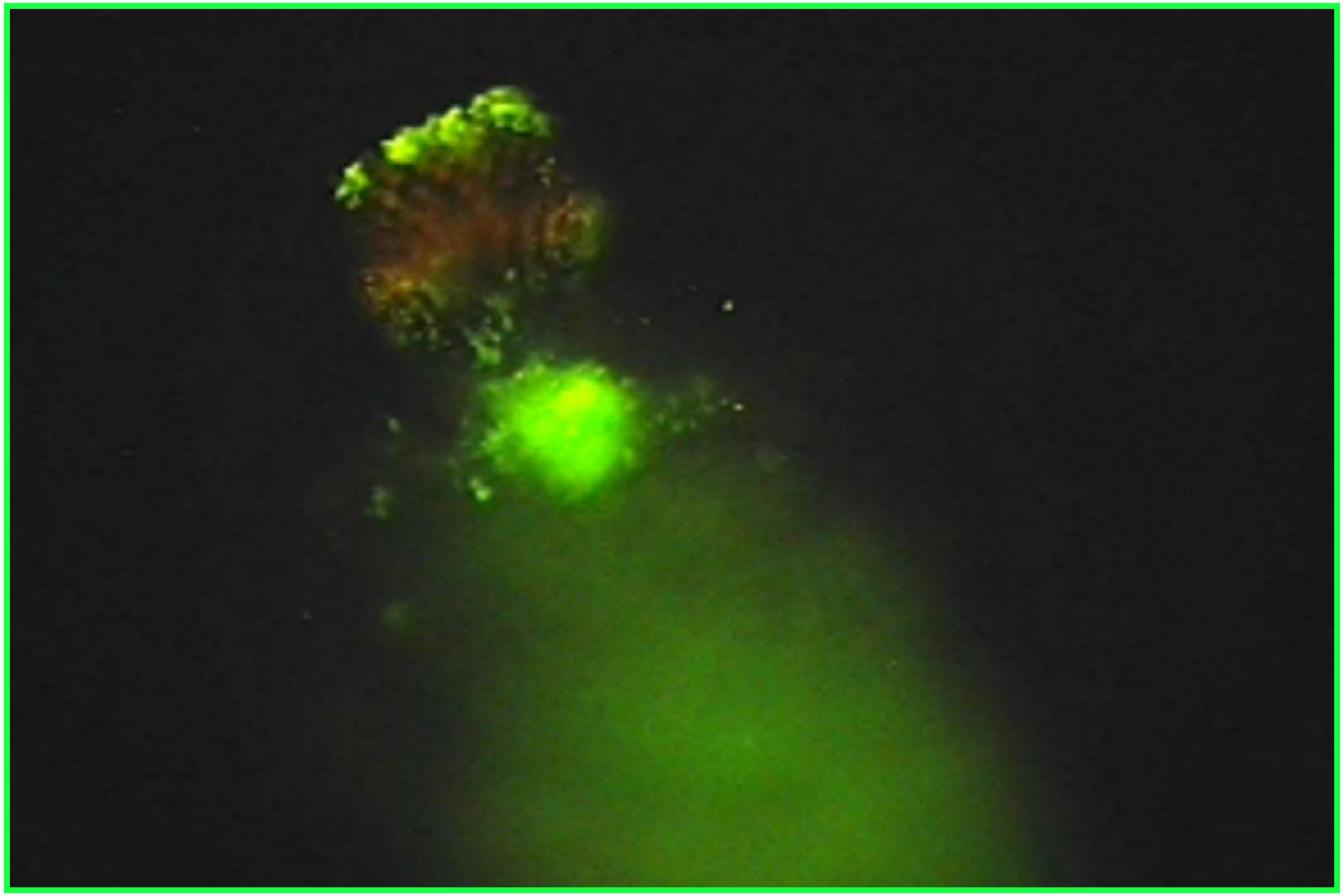


(Engelthaler et al. 2000)

Xenopsylla cheopis midgut and proventriculus infected with *Yersinia pestis*

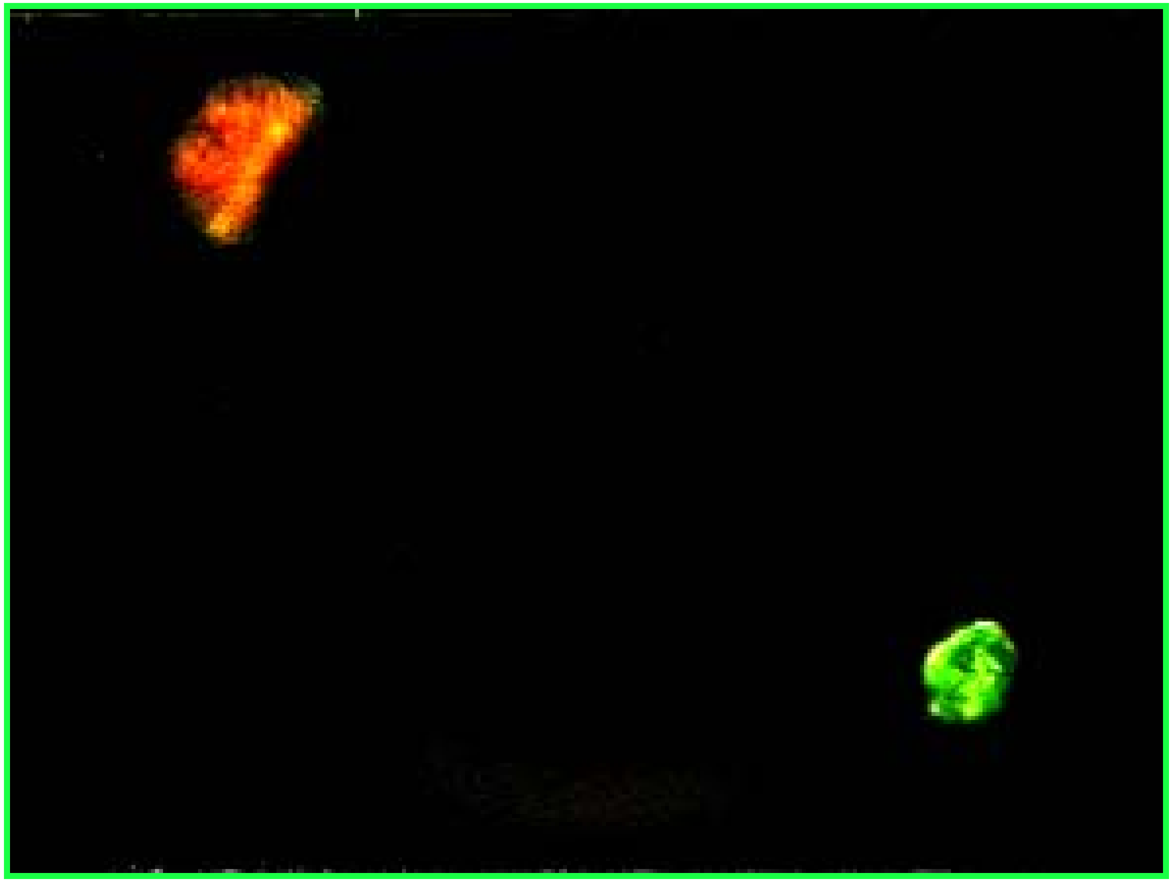


Xenopsylla cheopis midgut and proventriculus infected with *Yersinia pestis*

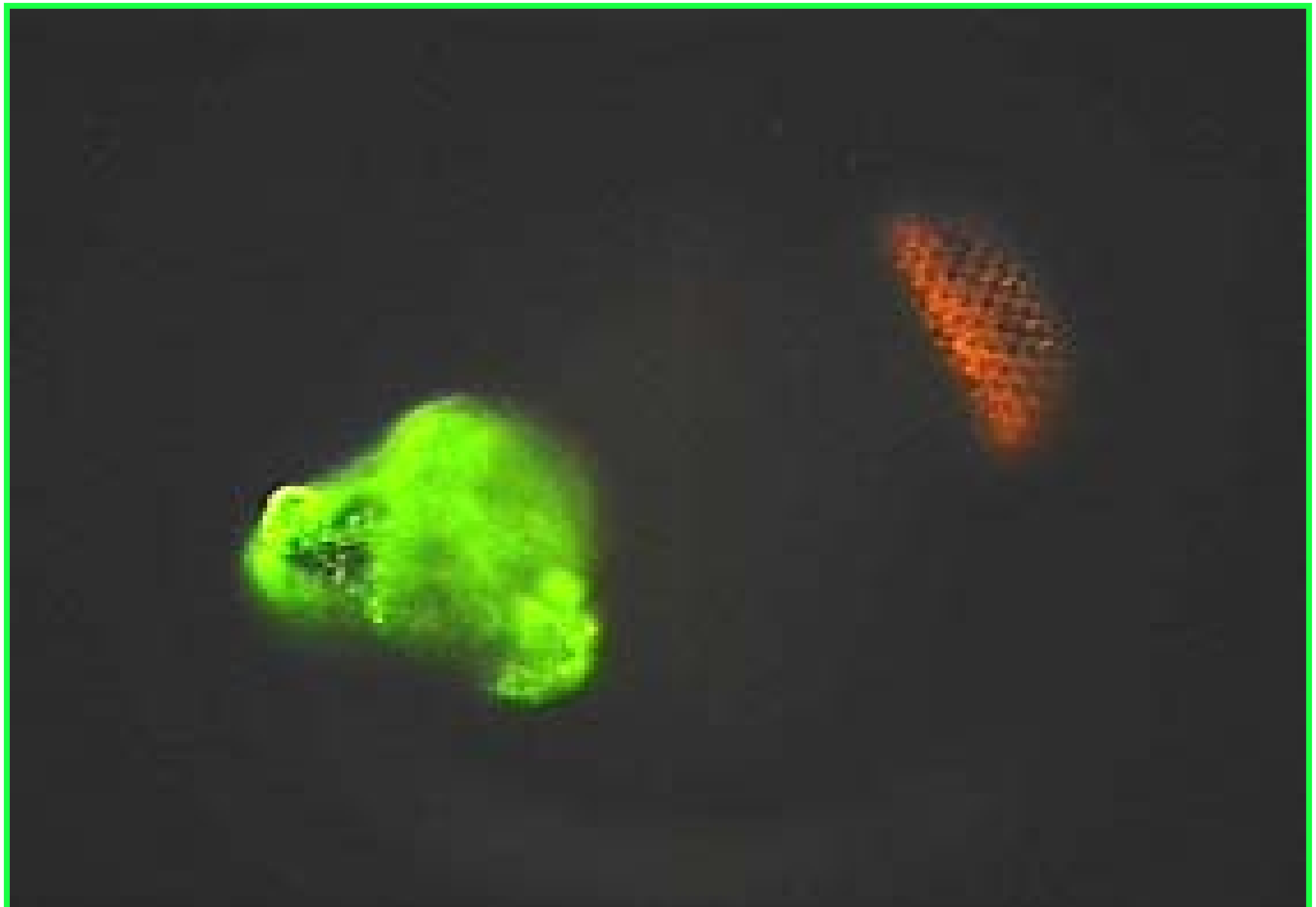


Oropsylla montana midgut infected with *Yersinia pestis*

Oropsylla montana midgut infected with *Yersinia pestis*



Oropsylla montana midgut infected with *Yersinia pestis*



QCPCR and GFP Results

- *X. cheopis* block and transmit more quickly
  - Exceptional vector – can block within 5 days and transmit at high frequency
  - *Y. pestis* simultaneously colonizes midgut and proventriculus

- *O. montana* are not efficient “blockers”, but can have a persistent midgut infection
  - Relatively inefficient vector, but may act as a reservoir
  - Mechanical transmission or transmission by partially blocked *O. montana* might be important

New Directions and Ideas

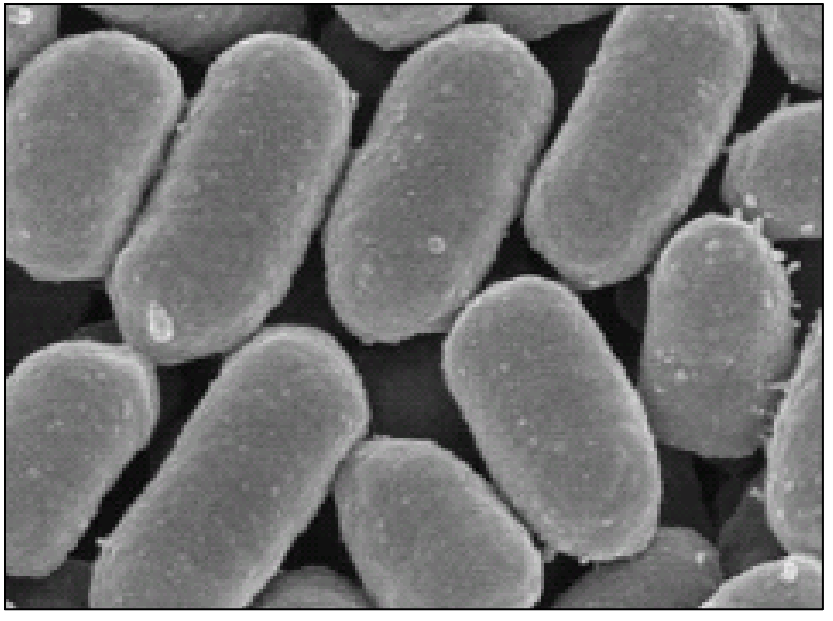
- Biofilms
- “Early Phase Transmission”

A Plague Biofilm?

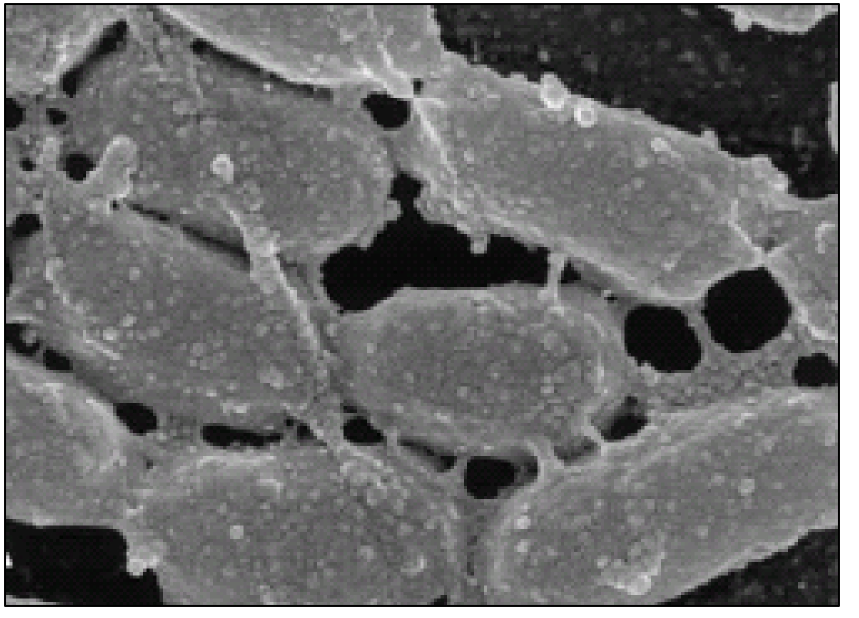
- Recent studies* have identified a biofilm produced by *pestis*
  - The *hms* genes are homologous to genes in other biofilm-forming bacteria
  - Allows bacteria to form dense aggregates in the midgut, surrounded by an extracellular matrix
  - Aggregates can adhere to the cuticle-covered spines in the proventriculus
  - Produced only at 28C, in the flea midgut
  - Appears to contain lipid derived from the flea blood meal

*Jarrett et al. 2004; Erickson et al. 2006; Forman et al 2006;

Biofilms
and the Hms Gene Locus



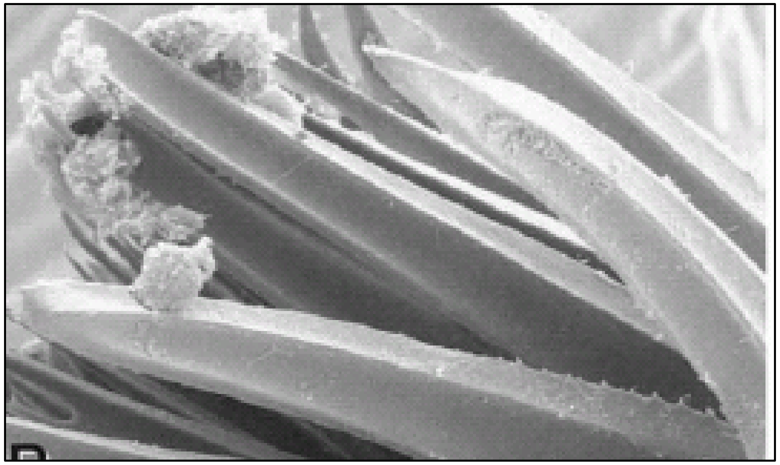
hms- Y. pestis



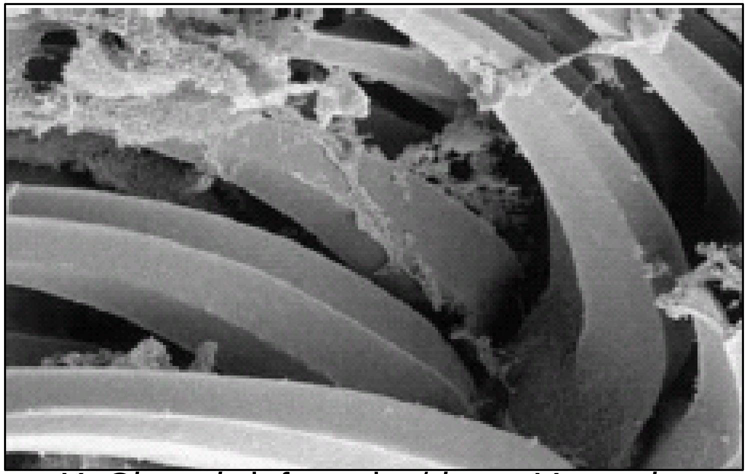
hms+ Y. pestis

Jarrett et al., 2004

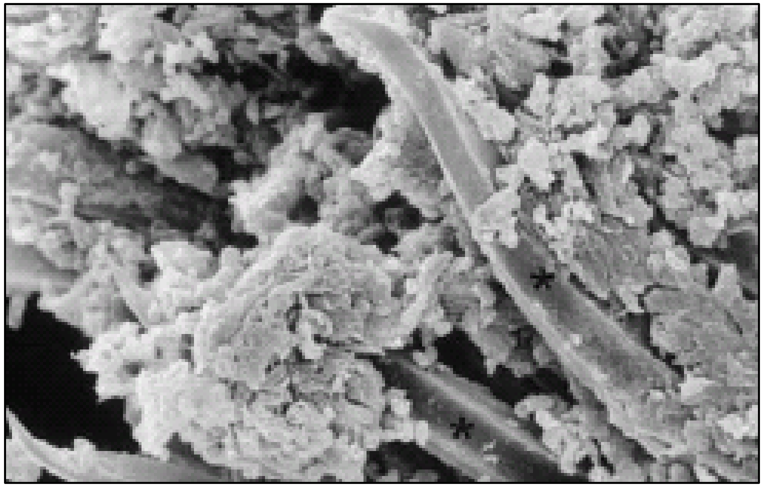
More Cool Pics



Uninfected *X. cheopis*



X. Cheopis infected w/ *hms- Y. pestis*



X. Cheopis infected w/
hms+ Y. pestis

Jarrett et al., 2004

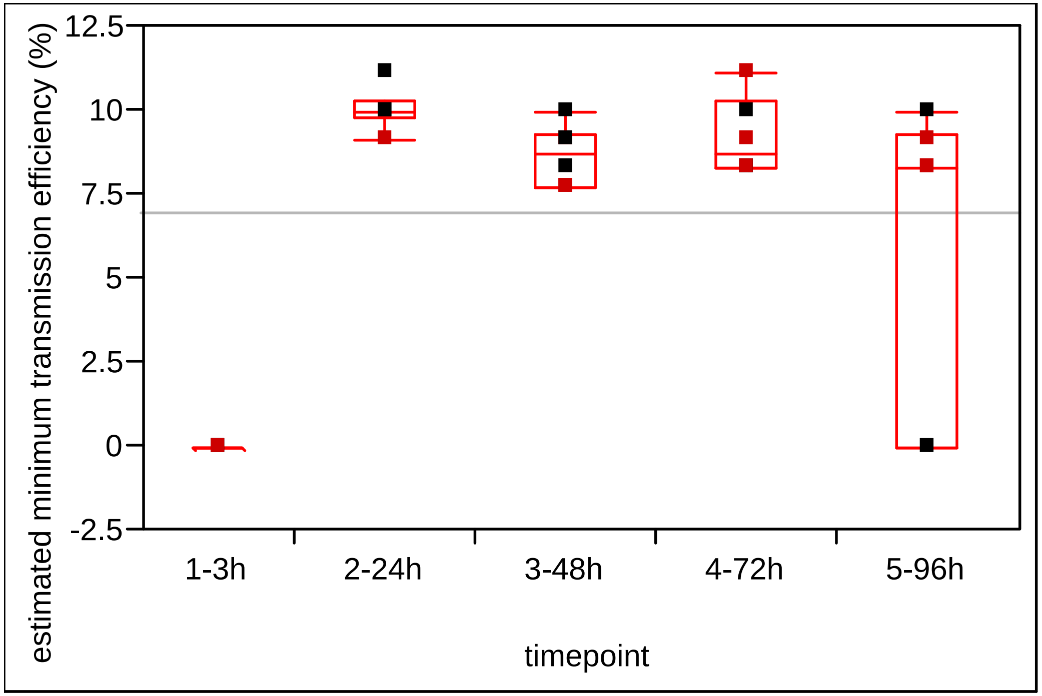
Evolving Thoughts on The Plague Dogma

A Changing Paradigm

- Do fleas really need to be blocked to transmit?
  - One *O. montana* in my studies transmitted on day 4 p.i. – too early for a block to develop
- Was this mechanical transmission?
- Might the old plague transmission dogma be insufficient?

"Early Phase Transmission" by *Oropsylla montana*

- Early-phase transmission of *Y. pestis* occurred reliably 1-4 d after infection.
- Block formation was not required to observe efficient transmission.
- The "extrinsic incubation period" (1 d) was much shorted than reported previously (10-53 d).



- High transmission efficiency coupled with EPT should lead to rapid spread in a susceptible host population.

Eisen et al. 2006

Other Research Avenues

- Antibiotic Resistance
  - Resistant strains found in Madagascar
  - Indiscriminate use of antibiotics will lead to more
- Climate and climate change
  - Recent modeling can help identify high risk years
  - Any changes will affect ecology of disease – for better and worse
- More genomics, transcriptomics, and proteomics
- Ecological niche modeling
- Improved molecular epidemiology
  - PH and Biodefense
- Vaccine Development

Final Thoughts

- *X. cheopis* is an unrivaled vector
  - Likely due to biological factors in flea rather than in *Y. pestis*
- New world flea-pestis dynamics represent a different life cycle, allowing for both rapid epizootic and prolonged enzootic cycles
- Recent discoveries are changing the Plague Dogma
  - “Early Phase Transmission”
  - The role of Biofilms
- Fleas act as reservoir, vector and pathogenesis collaborator

Final, Final Thoughts

- Although *O. montana* is less efficient vector than *X. cheopis* by standard measures, it may be more efficient for natural epizootic and enzootic maintenance
- The flea/pathogen dynamic is just that – *dynamic*
  - This vector/pathogen relationship is relatively new
  - And it looks like the beginning of a beautiful friendship

Thank You

www.TGenNorth.org